Dental macrowear, diet and anterior tooth use in *Piliocolobus badius* and *Colobus polykomos*

Research Thesis

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Abstract

Inferences about diet and feeding behavior from macrowear in extinct taxa hinge on the strength of associations between diet and dental wear patterns in extant taxa. Two similarly-sized colobine species living sympatrically in Ivory Coast's Tai Forest and that differ significantly in both diet and oral processing behavior provide an opportunity to explore the reliability of such associations. I tested the hypothesis that *Colobus polykomos'* aggressive processing of tough, hard *Pentaclethra macrophylla* pods manifests in greater anterior tooth wear relative to that observed in *Piliocolobus badius,* which does not exploit this resource. I assessed rates of anterior tooth wear in a sample of ten adult *C. polykomos* and nineteen adult *P. badius* naturally deceased specimens collected from the Tai Forest. I compared differences in ratios of dentin exposure to total occlusal area between anterior and posterior teeth of these species. Mann-Whitney *U* tests reveal that the ratios of averaged first and second incisor wear to first molar, second molar, and third molar wear in *C. polykomos* are significantly greater than those of *P. badius* for each interspecific comparison (*p*-values for Mann-Whitney *U* tests range from 0.036 to <0.001). These results indicate that known differences in diet, food material properties, and oral processing behaviors are reflected by increased incisal wear compared to molar wear in *C. polykomos* relative to that of *P. badius*. These results demonstrate the utility of incorporating multiple teeth and multiple tooth types for inferring diet and oral processing behavior in fossil primates.

Introduction

The Western Red Colobus, *Piliocolobus badius,* and the Western Black and White Colobus, or King Colobus, *Colobus polykomos,* are two similarly-sized species of African colobines. *Piliocolobus badius* is one of seventeen species of red colobus in the genus *Piliocolobus,* while *Colobus polykomos* is one of five species of black and white colobus within the genus *Colobus*. Both genera fall within the subfamily Colobinae, of the family Cercopithecidea. Colobines are characterized by their reduced or absent thumbs, multi-chambered stomachs capable of processing various toxins, deep jaws, narrow incisors, and high, sharp shearing crests on their bilophodont molars (Oates and Davies, 1994; Fleagle, 2013). In Africa, colobines live in warm, seasonal, tropical environments that produce a variety of food sources, allowing them to succeed as a diverse subfamily occupying many different ecological niches. The fossil record demonstrates that colobines have exhibited great behavioral and morphological diversity since the early Miocene. One of the ways this diversity can be recorded is through dental wear, the process by which food items and/or the act of chewing itself displaces or removes enamel from the occlusal surface of the tooth.

Different diets and/or chewing mechanisms leave unique patterns of wear on teeth. When correlated with observed dietary behavior in extant taxa, dental wear can be used to infer the diets of fossil primates. However, most studies of fossil dental wear have focused on signals from individual teeth in the dental arcade (Crompton and Hiiemae, 1970; Cuozzo and Sauther, 2015; Fiorenza, 2015; Fiorenza et al., 2011; Schmidt, 1998), neglecting behavioral signals that can be gained from interpreting differential wear across the dentition (Morse et al., 2013; Teaford, 1982). One of the most significant behavioral differences between *Piliocolobus badius* and *Colobus polykomos* is the latter's frequent and aggressive use of its incisors to process the tough and woody

Pentaclethra macrophylla seed pods (McGraw et al., 2015). At Taï, *Pentaclethra macrophylla* makes up just over 12% of *C. polykomos*' annual diet, and comprises over half of their diet during September when the seeds are most abundant (Korstjens, 2001). If this dietary behavior of *C. polykomos* is as idiosyncratic and significant as some authors have argued, then, given its presumed reliance on aggressive use of the anterior dentition to process this food, we hypothesize that *C. polykomos* will exhibit significantly greater relative wear on its incisors than *P. badius*. In this study, I provide data on the relative amounts of macrowear in *P. badius* and *C. polykomos* incisors and molars in order to assess the above hypothesis.

Figure 1: Images of the colobine species compared in this study. Left: *Piliocolobus badius*, the Western Red Colobus. Right: *Colobus polykomos*, the Western Black and White Colobus, or King Colobus. Photos taken in Taï Forest, Côte d'Ivoire by W. Scott McGraw.

Background

1. The Significance and Mechanisms of Dental Wear

Associating dental wear patterns with known diets in extant species is a powerful tool for inferring the diets of extinct taxa, as different diets and oral processing behaviors produce unique wear patterns (Janis, 1984; Ungar, 1988). Dental wear can be further divided into microwear, macrowear and mesowear. Microwear refers to the microscopic pits and scratches/striations on the

surface of a tooth resulting from tooth-tooth (attrition) or tooth-food contact (abrasion) (Imfeld, 1996). 'Scratches' or 'striations' are typically developed when tough, flat, and/or fibrous food items are processed in the diet, such as leaf stems and twigs, whereas 'pits' result during the pulping or shattering of hard or brittle food items with high relief contours, such as fruits, nuts, seeds, and buds (Scott et al., 2012). Macrowear refers to the overall deterioration of the occlusal surface of a tooth from attrition and/or abrasion, primarily manifesting in the elimination of enamel and gradually increasing the exposure of the underlying dentin (Schmidt, 2010). The third type of wear, mesowear, is a combined measurement of attrition and abrasion, which takes into account both cusp relief and cusp shape (Fortelius and Solounias, 2000). Fortelius and Solounias define cusp relief as the "difference in height between cusp tips and inter cusp valleys," whereas cusp shape describes how sharp, rounded or blunt the cusp is, with sharp cusps signaling highly attritive diets while blunt cusps form as a result of primarily abrasive diets.

Of the three types of wear, dental microwear is most commonly employed to infer fossil diets through comparing microscopic artifacts of known diets in living taxa with those found in fossil teeth (Scott et al., 2005; Lalueza and Perez-Perez, 1996). Comparing the microwear of extant species to that of extinct taxa allow for a direct inference of specific food types consumed by fossil forms. For example, higher proportions of pitting on the occlusal surface indicate hard/brittle foods in the diet, whereas a higher concentration of striations are the result of fibrous foods that require shearing by the teeth (Scott et al., 2012). One potential drawback of using microwear to infer diet, however, is the "last supper effect," in which microwear signals on the occlusal surfaces of teeth are continuously "erased" and replaced with microwear created by the most recent food item consumed (Grine, 1986). The last supper effect potentially limits our ability to infer the overall habits/lifestyle of an individual because only foods consumed late in life are preserved in

microwear. Macrowear, on the other hand, accumulates over the lifespan of the individual, and therefore provides a more comprehensive view of an individual's overall dietary and oral processing habits.

Mechanisms of Mastication

Traditionally, mastication is divided into two cycles: "puncture-crushing" and "chewing" (Crompton and Hiiemae, 1970; Hiiemae and Kay, 1972; Hiiemae, 1976). In puncture-crushing, teeth are used to puncture and chop food items without the molar occlusal surfaces necessarily contacting. This results in flat, blunted surface wear of molars. During chewing, molar occlusal surfaces contact one another during what is called the power stroke, which is divided into two phases (Hiiemae and Kay, 1972). In phase I of the power stroke, molar cusps shear across each other as the jaw moves medially and laterally, producing both lingual and buccal phase wear facets that eventually lead to an oblique sloping of the molar occlusal surfaces. During phase II, the jaw moves superiorly such that the upper and lower molar cusps crush and grind the bolus between them. Differences in the levels of puncture-crushing versus chewing required to process various food items will result in different overall patterns of macrowear on the molar occlusal surfaces. Hard and brittle foods, such as seeds and nuts, require more frequent 'puncture-crushing' motions of molars with relatively little need for tooth-tooth contact and/or shearing, resulting in more equal wear on the buccal and lingual cusps and therefore a flatter overall pattern of occlusal wear. Oblique occlusal wear is thus thought to be the result of frequent processing of more mechanically refined or tough foods such as leaves, which require a higher degree of chewing and shearing (Hiiemae, 1976; Hiiemae and Kay, 1972; Smith, 1984; Taylor, 1963).

The patterns and mechanisms of wear discussed above pertain to individual molars, and do not necessarily require comparisons with other teeth across the dental arcade, nor do they consider the effects of diet on the anterior dentition. While mastication and food processing is accomplished primarily through use of the molars in most species, the anterior teeth are largely involved in the ingestion and procurement of food items. The evolution of primate craniodental anatomy is characterized by a shortening of the face and jaws, which brought the incisors closer to the chewing muscles of the face, facilitating higher biting and chewing forces (Butler, 2000). The incisors of many primate species are adapted for specialized diet and ingestive behavior. For example, the anterior dentitions of marmosets and aye-ayes are narrow, sharp, and protruding to allow these species to gnaw through the bark of trees, whereas orangutans possess wide, shovel-shaped incisors adapted for the ingestion of fleshy fruits. The incisors of the two colobine species analyzed in this study are nearly identical, therefore a significant difference in the wear patterns of their incisors, if found, could have important implications for interpreting the function of generalized dentitions in the fossil record.

Morse et al. (2013) provided data on the differential wear across the molar rows of *Piliocolobus badius, Colobus polykomos,* and *Cercocebus atys* from the Taï Forest, emphasizing the fact that incorporating multiple teeth into studies of dental wear is necessary to infer differential tooth use. Morse et al. measured relative occlusal wear as the ratio of exposed dentin area to total occlusal area in the lower postcanine teeth. (Macrowear in the present study was quantified in the same way.) A few differences between the two colobines were found in terms of the relative wear gradient from M1 to M3, with *P. badius* showing overall higher amounts of absolute wear as well as higher variation in wear gradients between individuals across all three molars than in *C. polykomos.* While the authors demonstrate that dietary differences and differential tooth use can

result in varying wear gradients across the dental row, they do not identify/postulate specific differences in diet between the two colobines that may have resulted in these observed molar wear gradients. Instead, the authors focus on the clear differences in wear between *Cercocebus atys* and the two colobines species combined. The authors attribute the difference to *Cercocebus atys'* frequent consumption of the large and hard *Sacoglottis gabonensis* seed casings (Daegling et al. 2011; McGraw et al., 2011). Additionally, *Colobus polykomos'* frequent use of its incisors to process *Pentaclethra macrophylla* pods is only mentioned anecdotally, and the authors do not identify any signal of this behavior in the molar wear gradient of *C. polykomos*. Thus, while Morse et al. successfully demonstrate that incorporating comparisons of multiple teeth into studies of dental macrowear is necessary to reveal behavioral differences between taxa that studies of isolated molars fail to exhibit, the characteristic behavior of *C. polykomos* in processing *P. macrophylla* was not implicated in the wear of the molar teeth alone. It was therefore necessary to expand upon Morse et al.'s study to include comparisons of the anterior dentition, as these teeth play a crucial role in a significant proportion of oral processing behavior by *C. polykomos.*

2. Colobine Adaptations

In general, it is thought that cercopithecoids are adapted to folivory, with colobines having taken this adaptation a step further than cercopithecines (Delson, 1994). This dependence on folivory is evidenced by the colobines' multi-chambered stomachs and uniquely high shearing crests on their bilophodont molars (Oates and Davies, 1994). Bilophodont molars are associated primarily with a folivorous diet, as sharp shearing crests are necessary for breaking down tough leaves and extracting their nutrients (Lucas and Teaford, 1994). However, with the advert of more detailed field observation of colobine diets, as well as more detailed analyses of dental wear, it is

increasingly clear that seeds and other fruit items have played a larger role in colobine evolution than previously thought, and that leaves alone may not necessarily explain the dietary adaptations observed in extant colobines (Birchette, 1981; Davies, 1991; Delson, 1975; Happel, 1988; Korstjens, 2001; Thiery et al., 2017). For example, Happel (1988) argued that the bilophodont molars of cercopithecoids arose in response to seed-eating, rather than folivory. Happel points out that in seed-eating Old World monkeys, the space between lower molar lophs provides a stable surface on which to secure a small, round seed, allowing the cusps of the upper molars to efficiently crush the seed. Additionally, in nine of the twelve Old World monkeys Happel observed consuming fruits in captivity, the incisors were employed consistently to open the pericarp of the fruit and retrieve the seeds inside, similar to the observed incisor-dominated behavior of *C. polykomos*. Happel argued that while basal Old World Monkeys possibly evolved bilophodont molars for efficient processing of seeds, colobines later developed additional specializations for the incorporation of larger proportions of leaves into their diets (e.g. the multi-chambered stomachs and relatively higher shearing crests on the molars).

Indeed, there is much evidence in the fossil record for a largely granivorous and/or frugivorous radiation of stem cercopithecoids and later colobines. The colobines diverged from stem cercopithecoids sometime around 14-11 Ma, although a lack of fossils from this time period in this lineage make establishing this date difficult (Szalay and Delson, 1979). The genus most closely associated with stem cercopithecids, *Victoriapithecus,* which lived during the early-mid Miocene, is thought to have been adapted for terrestrial frugivory (Delson, 1994). In the last month, the discovery of new fossil Old World monkey teeth belonging to the genus *Alophia* was reported from a 22-million-year-old deposit in Nikwai, Kenya (Rasmussen et al., 2019), dating to even earlier than *Victoriapithecus*. The molars of this individual are cusped, and lack the bilophodont

morphology that characterizes all extant Old World monkeys. The authors suggest that these dental remains indicate that the earliest Old World monkeys were hard-object feeders, and that bilophodont molars evolved later in Cercopithecoidea evolution. The oldest known colobine, *Microcolobus,* which lived during the Late Miocene, is also thought to have been less reliant on leaves than extant colobus monkeys. *Microcolobus,* while possessing bilophodont molars, had lower molar cusps than those of living colobines or even the extinct European colobine *Mesopithecus.* Because of *Microcolobus'* primitive dental morphology and small size (4-5kg), this species is thought to have been an arboreal frugivore (Fleagle, 2013). Terrestrial frugivory is observed in the earliest well-known European colobine, *Mesopithecus.* Thiery et al. (2017) used microwear, relative enamel thickness and dental topography to show that *Mesopithecus* was likely an opportunistic feeder that often consumed challenging foods like seeds and nuts. Additionally, the high degree of wear on the bilophodont molars of *Cercopithecoides* from Koobi Fora, dating to between 3 and 1.9 Ma, shows strong evidence for the processing of gritty foods found on the ground (Birchette, 1981). However, several other fossil colobines of the same time period are thought to have lived in the trees, where such resources are less accessible. For instance, Pliocene fossils *Rhinocolobus turkanaensis* and *Paracolobus chemeri* from the Turkana Basin are considered to have been large, arboreal taxa living in a wooded savanna environment (Leakey, 1982; Delson and Dean, 1993). Leakey (1982, 1987) has suggested that these arboreal African fossil colobines are most closely related to extant colobines, based on arguments that living primates are specially adapted to folivory, while the terrestrial African fossil colobine *Cercopithecoides* is most closely related to the earlier, extinct European seed-eating colobines like *Mesopithecus* (Leakey, 1982; 1987). The fossil record as described above indicates that African colobines evolved in response to many different dietary pressures, and that they have only

relatively recently become a primarily folivorous radiation. For this reason, and because dental wear is one of the most frequently employed tools for inferring the diets of fossil individuals, a motivation for the present study is to broaden our understanding of how diet and oral processing behavior is reflected in patterns of dental wear.

3. The Diets and oral processing behavior of *Piliocolobus badius* and *Colobus polykomos*

Piliocolobus badius and *Colobus polykomos* populate much of West Africa, with field observations detailing their diets coming from many localities, including Tiwai Island in Sierra Leone and the Taï Forest of Côte d'Ivoire, where the specimens of the present study were collected. Davies (1999) reports dietary observations for *Piliocolobus badius* and *Colobus polykomos* from Tiwai Island. According to this field study, *C. polykomos* consumed more seeds, young leaves, and mature leaves than *P. badius,* which incorporated about 17% of their diets from whole fruits and flowers. In both colobine species, almost half of their diets were non-foliar items, such as seeds, fruits and flowers, with seeds comprising at least five times the amount of non-seed fruit items in both species. While both colobine species consumed relatively large amounts of nuts and seeds, *C. polykomos* focused more on fully developed, thick and woody seed pods like those of *Pentaclethra macrophylla*, whereas *P. badius* primarily processed younger, less durable seed pods. A few years earlier, Georgina Dasilva (1994) also published dietary data on *C. polykomos* from Tiwai*,* noting that *Pentaclethra macrophylla* seed consumption in this species is significantly correlated with *Pentaclethra macrophylla* availability, while leaf consumption (both mature and young) is inversely correlated with seed availability. These results suggest that *C. polykomos* consumes leaves only when seeds are not available, contradicting the traditional view that

colobines are a primarily folivorous radiation (Hill, 1964; Napier, 1970). In the Taï Forest, dietary proportions of *P. badius* and *C. polykomos* are similar to those observed at Tiwai, with *P. badius* consuming 75% leaves and buds compared to 47% in *C. polykomos* (McGraw et al., 2015). According to Korstjens (2001), *Pentaclethra macrophylla* makes up about 12.7% of *C. polykomos'* annual diet on average, and up to 54.7% of their diet during September when the seeds are most abundant. In addition to dietary preferences, McGraw et al. (2015) reported oral processing data for the two colobines including frequencies of mastication and incision per ingestive event. When all ingested food items were considered, *P. badius* chewed 17.5 times per ingestive event, while *C. polykomos* chewed 20.4 times per ingestive event. Incisive events differed between the species as well, with *C. polykomos* averaging 3.09 incisions per ingestive event and *P. badius* incising 1.68 times per ingestive event, when all foods are considered. This difference in incision frequency arises primarily due to the unusually high number of incisions required to processes/open a *Pentaclethra macrophylla* pod, which yielded counts of up to 27.7 incisions per ingestive event in *C. polykomos.*

Pentaclethra macrophylla seeds, also called African oil bean seeds, are roughly the size of a quarter, and are encased in large, hard, fibrous boomerang-shaped pods (McGraw et al., 2015). The pods are fibrous on the outside, with a homogenous matrix beneath, and are about one third as tough as tree bark. The seeds inside are not hard or brittle; rather, both the seed coat and seed flesh are within the toughness range of fleshy fruit (Constantino et al., 2009; McGraw et al., 2015; Vogel et al., 2008). Processing the *P. macrophylla* pods to acquire the fleshy seeds is a laborious task for *C. polykomos,* requiring up to 15 minutes per pod (McGraw et al., 2015). *C. polykomos* will typically secure itself in a seated perch, hold onto the pods with both hands, and pull on the fibrous exterior of the pod with their anterior teeth using great force in order to expose the fleshy

seeds inside (McGraw et al., 2015). *C. polykomos*' ability to consume and digest these seeds is an enigma. While the seeds present several nutritional benefits like high protein content and essential fatty acids, the unfermented seeds also contain poisonous alkaloids and sometimes harbor harmful bacteria (Akah et al. 1999).

Figure 2: *Pentaclethera macrophylla* pods from the Taï Forest. These hard, tough and fibrous pods are difficult to process, which is thought to explain *Colobus polykomos'* frequent use of its incisors during food processing. Photos by W. Scott McGraw.

4. Purpose of This Study and Hypotheses

The purpose of this study is to determine whether *Colobus polykomos'* frequent and aggressive use of its incisors to process *Pentaclethra macrophylla* pods results in significantly different patterns of wear across the dental arcade between the two colobine species. Specifically, I sought to determine whether this behavior results in a higher degree of wear on the incisors of *C. polykomos,* with the level of incisor wear for each individual scaled to the wear on the molars. The scaled measure of incisor to molar wear reflects that rate at which incisors wear relative to molars. Such a difference in incisor wear rate could potentially provide a new signal for identifying this oral processing behavior in fossil species. As mentioned previously, most studies of dental wear in fossil taxa focus on signals provided by individual teeth (Crompton and Hiimae 1970; Cuozzo

and Sauther 2015; Fiorenza 2015; Fiorenza et al. 2011; Schmidt 1998), whereas the methods outlined here demonstrate the utility of comparing wear across the dental arcade for identifying differences in diet and oral processing behavior (Morse et al., 2013; Teaford, 1982). This exploration is particularly important for the colobine radiation, as the dietary evolution of colobines is still largely debated. We predict that *C. polykomos*' frequent and aggressive use of incisors during processing of *P. macrophylla* pods will result in a higher degree of wear on its anterior dentition relative to that on its molars when compared to *P. badius,* which does not consume *P. macrophylla* seeds.

Figure 3: *Colobus polykomos* individuals collecting and processing *Pentaclethera macrophylla* pods. Photo by W. Scott McGraw.

Materials and Methods

This study involved ten adult *C. polykomos* and nineteen adult *P. badius* maxillae and mandibles from naturally deceased specimens collected by members of the Taï Monkey Project from Tai National Park. These specimens are currently housed in the Department of Anthropology at The Ohio State University. Anterior dentitions were photographed with a Leica DMS 1000 microscope, situated under the lens such that the occlusal surfaces of the teeth were parallel with

the camera's field of view. ImageJ was used to measure the two-dimensional area of exposed dentin and the total occlusal area on each tooth, using digital millimeter scales produced by the Leica microscope. These measurements were used to calculate relative degree of wear for each tooth by dividing the area of exposed dentin by the total occlusal area. Incisor wear measurements were taken by averaging the relative wear for all upper and all lower incisors (central and lateral) that were present in each specimen (which varied by individual in number and subset of incisors present). This produced two values of incisor wear (upper and lower) for each individual in cases where incisors were present in both jaws (many specimens lacked incisors in one of the jaws, or one of the jaws was missing altogether). Data on relative molar wear are from Morse et al. (2013), who used a Pentax K100D digital camera to obtain high resolution photos of the mandibles. This was done by positioning the occlusal surfaces parallel to the camera's field of view, with a millimeter scale included level with the occlusal surfaces of the teeth. Morse et al. analyzed one side of the lower dentition for each specimen, selecting the side that contained all four postcanine teeth (P4, M1, M2, M3). In cases when both sides of the mandible contained all four postcanine teeth, the analyzed side was chosen at random.

To calculate relative incisor wear for each individual, upper and lower average incisor wear was divided by the degree of wear for each of the specimen's three mandibular molars, producing six measurements per individual in cases where incisors were present in both the upper and lower jaws (i.e. upper I's to lower M1, upper I's to lower M2, upper I's to lower M3, lower I's to lower M1, lower I's to lower M2, and lower I's to lower M3). Nonparametric Mann-Whitney U-tests for comparing two samples with no specific distribution were conducted in SYSTAT to compare the six subsets of incisor to molar wear ratios between the two species.

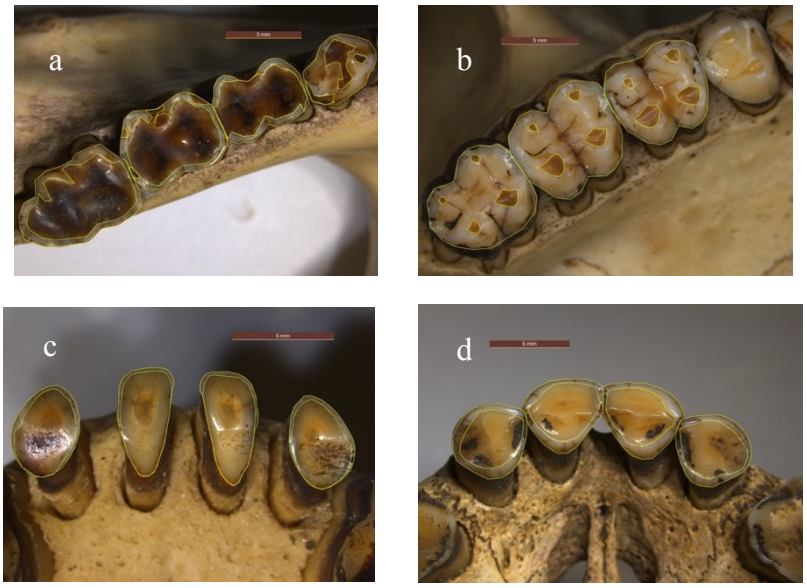


Figure 4: Examples of images used to calculate wear ratios with ImageJ. Yellow outlines represent exposed dentin and total occlusal surface. (a) Mandibular incisors of *P. badius.* (b) Maxillary incisors of *C. polykomos.* (c) Mandibular molars of *P. badius.* (d) Maxillary molars of *C. polykomos.*

For each specimen, incisor wear was divided by the wear of each of the specimen's molar wear value in order to produce a scaled measure of relative wear. This scaling step was important because our goal is to quantify the wear *rate* of the incisors relative to the rate of molar wear in each individual. Additionally, as quantified in Morse et al. (2013), the molars of *P. badius* are, on average, more worn than those of *C. polykomos.* Consequently, absolute incisor wear from these samples alone is not comparable. We lack data for the ages at death for the individuals used in this study; therefore, it is not possible to rule out the possibility that the higher absolute degree of wear in the *P. badius* specimens is due to age bias. Our sample does not represent a cross section of the population because it is comprised of specimens that had died naturally, likely at an old age. If indeed there is a significant difference in average age between the two colobine samples- with the *P. badius* individuals having a higher age at death- then the similarity of molar and incisor wear rate observed in this species could be the result of the molars having had more time to wear after the incisors had reached maximum wear (i.e. total dentin exposure), rather than being an authentic reflection of behavior differences. Such an age bias has the potential to mask any wear gradients that may have been present earlier in life. While it is important to address possible sampling biases

like this, we have no reason to suspect that there is a fundamental difference in the distribution of wear stages between the two samples, as there was no systematic mechanism for collecting and/or selecting the specimens that were used in this study. Furthermore, in most of the *P. badius* individuals, an enamel ring was still present around the molar and incisor occlusal surfaces, indicating that the teeth were not yet completely worn. This means that despite the higher overall degree of wear observed in the *P. badius* specimens, it can be assumed that our scaling method for measuring incisor wear relative to molar wear eliminated any non-behavioral biases that may exist between the two colobine samples.

Error Analysis

Inter-observer error was calculated to verify consistency between the methods conducted in this study with those of Morse et al. (2013). The error study was performed by repeating Morse et al.'s measurements of sixteen mandibular molars from three different individuals, using the method and materials utilized in the present study. For each of the molars, I divided my measurements of dentin area to total occlusal area ratios by the ratios calculated by Morse et al. for each of the sixteen molars, and subtracted this proportion from one to obtain a fraction representing the amount of discrepancy between my measurements and the measurements presented in Morse et al. (2013) for each molar. The absolute values of these fractional discrepancies were taken and averaged, yielding a mean inter-observer error of 8.62%.

Intra-observer error was calculated by repeating measurements for ten incisors from a total of two individuals, for one of which only the upper incisors were used and for the other, both upper and lower incisors were used to calculate error. The measurements were repeated on a later day from which the original measurements were taken, and all steps from mounting, photographing,

and using ImageJ to measure the relative wear were replicated. Intra-observer error was calculated in a similar fashion as inter-observer error, by dividing the repeated measurements by the original measurements, and taking the absolute values of fractions produced when subtracted from one. This process yielded an intra-observer error of 1.91%.

Results

Each of the six comparisons of relative incisor wear showed statistically significant differences between the two colobine species ($p < 0.05$). These results are summarized in **Table 1** and **Figures 5** and **6**. The wear ratios for *P. badius'* upper and lower incisors to each of the three lower molars are all close to and slightly above one, indicating that the incisors and molars of *P. badius* wear at roughly the same rate. In *C. polykomos*, the steeper positive regression from first to third molars is likely an artifact of the molar eruption sequence, in that the first molar erupts first, allowing it more time to wear relative to the second and third molars (Harvati, 2000). Nevertheless, the incisors of *C. polykomos* are approximately three times more worn than even the first lower molar.

As documented in Morse et al. (2013) and discussed previously, the molars of *P. badius* are generally more worn in absolute terms than those of *C. polykomos.* The same is true for the incisors, which is why the incisor to molar wear ratios for this species are all close to one. While this difference in absolute wear between samples prompted us to consider whether our data were skewed by age, we are confident that our scaling method successfully produced comparable samples. I therefore infer from our results that there is an authentic behavioral difference captured by the wear gradient data.

The data indicate that the behavioral differences between the two colobine species manifest in a significantly different wear pattern: *C. polykomos'* aggressive and frequent use of its incisors to process *P. macrophylla* pods results in a faster rate of incisor wear. This represents an additional behavioral/dietary signal in the wear of these specimens to those identified by Morse et al. (2013), demonstrating the importance of understanding relative anterior tooth wear. Because this difference is statistically significant within upper and lower incisors, this particular pattern of wear of more heavily worn incisors than molars across the dental arcade may be used to identify similar food processing behaviors in the fossil record as that carried out by *C. polykomos*.

Incisor Group	Position of molar	*C. polykomos*	*P. badius*	p-value
Upper	M1	3.33	1.25	0.036
	M2	4.77	1.42	0.002
	M3	8.00	1.60	0.005
Lower	M1	3.88	1.00	0.003
	M2	4.58	1.09	0.000
	M3	7.23	1.16	0.000

Table 1: Values of relative incisor wear divided by relative molar wear for the upper and lower incisors, and each of the three lower molars, for *C. polykomos* and *P. badius*. Values represent the fraction of exposed dentin on the incisor occlusal surfaces, divided by the fraction of exposed dentin area on the mandibular molar occlusal surfaces.

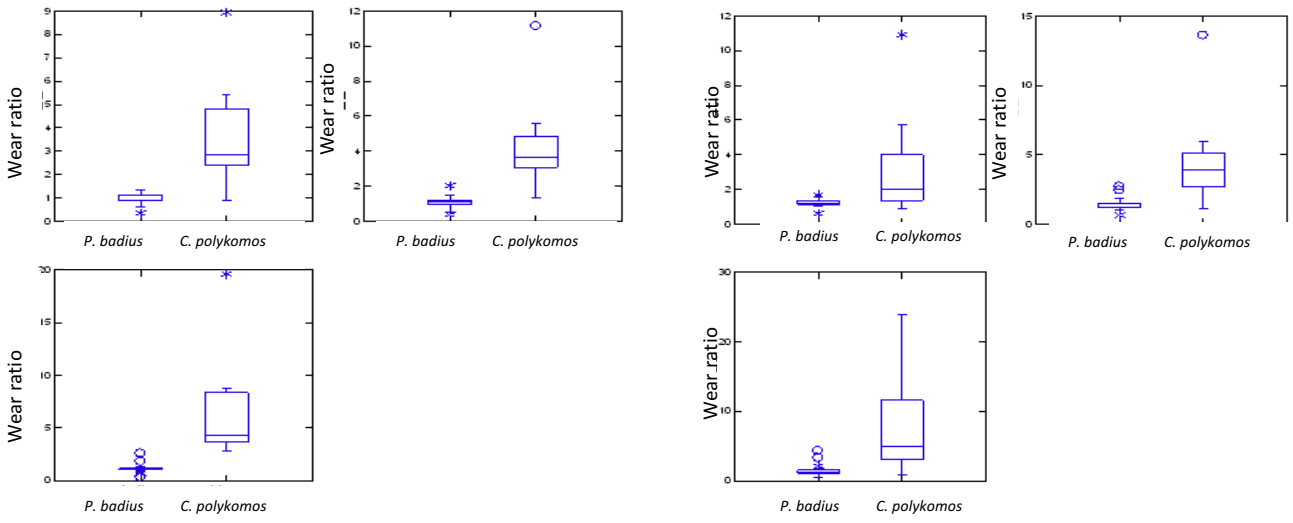
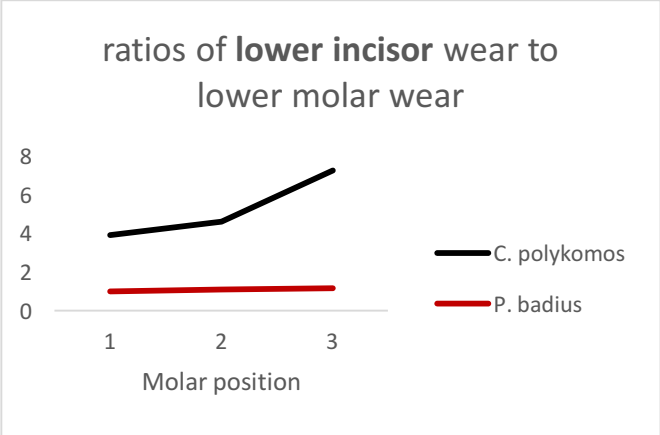


Figure 5: Box plots produced in SYSTAT showing results of the six different measurements of relative incisor wear for lower (left group of plots) and upper incisors (right group of plots), compared to the lower M1, M2, and M3. All six comparisons yielded significantly different values for relative incisor wear between the two colobine species.



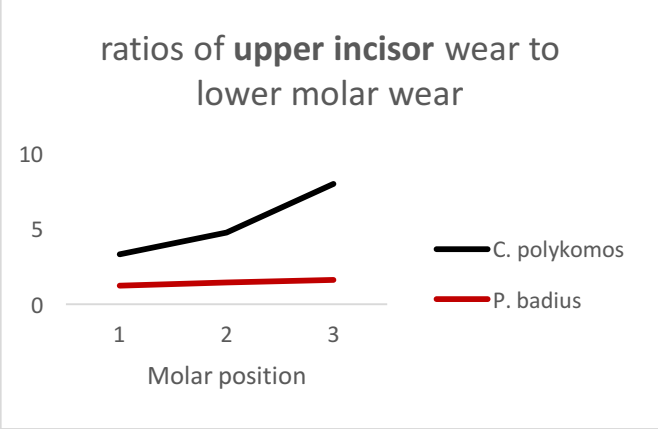


Figure 6: Graphical representation of the data presented in **Table 1.** The data show that *P. badius* wears its incisors at close to the same rate as it wears it molars, whereas *C. polykomos* wears its incisors much faster than its molars. The regression has a positive slope due to the eruption sequence of molars. Because the first molar erupts first, it has more time to wear, and is thus closer in relative wear to the incisors than the other two molars, which erupt later in life (Harvati, 2000).

Discussion

The results of this study demonstrate that *C. polykomos*' idiosyncratic oral processing of *P. macrophylla* pods involving aggressive incisor use is reflected in its incisor wear. In both study taxa, the incisors wear mostly through enamel removal on the lingual side of the incisors. Unlike colobines, cercopithecines, generally do not have enamel on the lingual side of their incisors. This is thought to be an adaptation to their largely frugivorous diet, as the lack of lingual enamel creates sharp incisal edges to facilitate the cutting and scraping of fleshy fruits in order to reduce them prior to mastication with the molars (Shellis and Hiiemae, 1986). According to Shellis and Hiiemae (1986), the colobines' possession of enamel on both the labial and lingual sides of the incisor creates a blunt incisal edge, which reduces the amount of force and stress that can be concentrated in one location, thus facilitating the gripping and tearing of leaves. The results of this study, combined with data on *C. polykomos*' reliance on and exploitation of *P. macrophylla*, raise questions about whether *C. polykomos*' dental morphology may be uniquely adapted to processing

this food item. It is possible that the removal of lingual enamel through *C. polykomos'* frequent processing of *P. macrophylla* pods increases *C. polykomos*' efficiency in this incisor-dominated behavior later in life, forming sharper incisors like those of more frugivorous cercopithecines. Additional study of colobine incisor functional morphology and oral processing across *C. polykomos*' lifespan is needed to test this hypothesis.

Interestingly, *C. polykomos* treats the *P. macrophylla* pod similarly to the way cercopithecines are known to process fruits: by holding the food item with their hands and reducing the item with the incisors before mastication with the molars (McGraw et al., 2015). This overlap in behavior between the two subfamilies is not a predicted outcome of a study done by Walker (1976), who contrasts the colobines and cercopithecines in terms of their diet based on differences in the amount and orientation of microwear striations on the incisors. Specifically, the colobines possessed fewer striations overall, and most of their striations were parallel with the incisal edge, whereas cercopithecines had more striations on their incisors, mostly perpendicular to the incisal edge. The striations parallel to the incisal edge are thought to be the result of colobines using their blunt, relatively less sharp incisors to grab and drag leaves between the upper and lower incisors in order to separate the softer, parenchymatous portion of the leaf from the tough fibrous portion. By contrast, the perpendicular striations found on the incisors of cercopithecines are suggested to be the result of repeated piercing of fruit items. The sharpness of the cercopithecine incisors due to the lack of enamel on their lingual surface provides a less efficient route for leaf consumption than the blunt incisors of colobines, requiring several bites or slices to achieve the same goal. This fact likely explains Walker (1976)'s observations of striation differences between the subfamilies. While the cercopithecines were found to have higher percentages of striations that were perpendicular to the incisor edge (~60%), colobines too had nearly 30% of their striations oriented

in this direction. By contrast, the difference between the two primate groups in terms of striations parallel to the incisal edge was more marked. Parallel striations made up almost 60% of the colobine incisal striations, but comprised less than 10% of the cercopithecine striations. Walker argues that his results affirm a difference in diet and habitat preference between the two subfamilies; however, the relatively larger overlap between the two groups in terms of their number of striations resulting from frugivorous habits emphasizes the diversity of colobine diet and resultant wear signals.

Colobines, while possessing some specialized features such as multi-chambered stomachs and sharp molar shearing crests, are fairly generalized and lack specializations on the incisors that would suggest idiosyncrasies in their dietary and/or oral processing behaviors (Oates and Davies, 1994). Additionally, Daegling and McGraw (2001) found no significant biomechanical signals in the mandibles of *C. polykomos* and *P. badius* that correspond to known differences in diet and oral processing behaviors between these species. Daegling and McGraw predicted that *C. polykomos,* because they consume the tough and fibrous *P. macrophylla* pods, would have a more robust mandibular corpus than *P. badius*. Instead, the reverse was true, emphasizing the fact that diet alone is not always a sound predictor of biomechanical skeletal morphology. Additionally, Morse et al.'s comparative study of dental wear gradient between the Taï cercopithecids did not produce a unique signal for the significant dietary and behavioral differences between *P. badius* and *C. polykomos* that are explored in the present study. The results of both of the aforementioned studies demonstrate the importance and relevance of the results reported here, as we present a new metric through which *C. polykomos*' frequent and aggressive use of its incisors can be detected in its dental wear.

The present study relied on comparison of more than one taxa. Because fossil specimens are typically isolated and therefore lack additional specimens for comparison, inferring diet in fossils with this wear pattern could prove challenging and potentially inconclusive. Nevertheless, these results show that incisor-dominant behavior can be reflected in the relative wear on an individual's incisors, indicating that the anterior dentition is a crucial region to gather clues of specialized feeding behavior in extinct taxa that may otherwise not be represented in molar wear or skeletal/biomechanical morphology. More work is needed to identify additional correlates of incisor-dominated behavior so that this element of feeding can be more confidently inferred in fossil species.

The results of this study spark several additional questions for further research. For example, this study compared wear of incisors and molars, but did not include analysis of the canines or premolars. McGraw et al. (2015) mention that they were unable to confidently and consistently discern whether the canines were employed during processing of *P. macrophylla* pods, and thus denoted each event in which the anterior teeth were used as an "incision." It would therefore be useful to determine how wear on the canines of *P. badius* and *C. polykomos* differ, if at all, and whether the use of canines for processing this food item can be determined through analysis of wear. Another potential direction for future work includes determining whether *C. polykomos*' processing of *P. macrophylla* seeds increases the functionality of their incisors, or if the molars of *C. polykomos* possess adaptations to mastication-induced wear, such as thick enamel, that *P. badius* does not possess. Wear has been shown to be adaptive to certain oral processing behaviors (Pampush, 2016; Ungar, 2000), therefore it is possible that the frequent use of incisors by *C. polykomos* to open *P. macrophylla* pods serves to sharpen the incisors, making them more effective in processing this food throughout life. To test this, we would need field observations of

C. polykomos individuals of different ages in order to determine whether the species' efficiency in processing the pods increases throughout the lifespan. Lastly, it could prove useful to determine whether the omnivorous and mostly-folivorous diets (as is the case for *C. polykomos* and *P. badius*, respectively) differ in terms of their microwear signal. Therefore, a potential extension of the present study could involve comparing the striations on the incisors of *P. badius* and *C. polykomos* in a manner similar to that in Walker's 1976 study.

Conclusion

This study showed that the known differences in diet and oral processing behavior between *Colobus polykomos* and *Piliocolobus badius* manifest in a significant difference in wear of the incisors relative to each of the mandibular molars. Historically, colobine monkeys are generally categorized as folivorous primates, even though numerous observational studies have shown that colobines have diverse diets, both inter- and intraspecifically (Dasilva, 1994; Davies, 1999; McGraw et al., 2015). To this end, many authors have suggested that the bilophodont molars of colobines, which are traditionally argued to be adaptations to folivory, may actually have evolved in response to seed-eating during colobine evolution (Happel, 1988; Lucas and Teaford, 1994; Thiery et al., 2017). Additionally, colobines are known to have had a broad evolutionary history, with fossils having been found in a variety of habitats, representing a variety of body sizes, dental morphologies, and substrate choices. Colobine features therefore likely evolved in response to a diversity of dietary and oral processing behaviors, resulting in the relatively generalized radiation of monkeys observed today. Our results demonstrate a unique method by which dental wear can be used to detect dietary diversity among otherwise similar taxa, and reflect the importance of incorporating multiple teeth and multiple tooth types into studies of dental wear.

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